Effects of a *Spirulina platensis*-based diet on zebrafish female reproductive performance and larval survival rate

by

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Received: 2 Apr. 2012
Accepted: 20 Feb. 2013
Editor: E. Dufour

Key words

Cyprinidae
Danio rerio
Zebrafish
Reproduction
Spirulina platensis
Nutrition
Larvae

Abstract. – The zebrafish (*Danio rerio*) is a valuable laboratory animal model that is used in many research areas. However, little is known about the factors that can affect zebrafish reproduction. To determine the potential benefit of an *n*-6 fatty acid (FA)-rich alga on zebrafish reproduction endpoints, we investigated the effects of the cyanobacterium *Spirulina platensis* delivered as sole food for zebrafish broodstock. Compared with a commercial flake diet-fed group (FG), egg production of the *S. platensis* diet-fed group (SG) was lower. However, egg survival rate was significantly higher for SG (73%) than for FG (55%). Moreover, larvae from SG developed more quickly compared with larvae from FG, because the hatching time was significantly shorter in SG than in FG eggs. No difference in egg and/or larval weight and size was observed between treatments. Interestingly, two different maternal investment strategies were observed according to the diet type, emphasizing a tradeoff between egg quality and egg quantity. A *S. platensis*-based diet was well suited to meet larval dietary requirements, as evidenced by a larval survival rate of 69% at 31-days post fertilization. Therefore, based on the results of this study, a *S. platensis*-based diet is recommended for zebrafish larvae in the first few days of life.

Résumé. – Résultats d’un régime à base de *Spirulina platensis* sur les performances reproductives du poisson zèbre femelle et taux de survie des larves.

Le poisson zèbre (*Danio rerio*) est un vertébré modèle de plus en plus utilisé à des fins d’études toxicologiques, car il présente de nombreux avantages que ce soit en termes d’élevage ou de suivi du développement. Malgré le nombre considérable d’études dont il a fait l’objet, très peu portent sur les facteurs susceptibles d’influencer la reproduction de ce poisson, tant sur le plan comportemental que sur l’investissement maternel lié à l’apport nutritionnel. Dans un premier temps, cette étude a eu pour but de comparer le succès reproducteur des individus nourris avec deux types de nourritures aux propriétés sensiblement différentes. Les poissons ont été soit nourris avec de la spiruline, une cyanobactérie riche en oméga-6, soit avec des flocons commerciaux. Le second objectif de cette étude a été de comparer la survie larvaire en fonction de différents régimes alimentaires, dont certains à base de spiruline. Les résultats indiquent que la quantité d’œufs produite par les reproducteurs nourris exclusivement avec de la spiruline est 4,5 fois inférieure à celle des individus nourris avec des flocons. Néanmoins, la survie des œufs est significativement plus élevée chez les individus nourris avec de la spiruline (73%), comparativement à la survie des œufs produits par les reproducteurs nourris aux flocons (55%). Cette différence ne peut être attribuée aux caractéristiques biométriques des œufs qui sont semblables. Ces résultats témoignent d’un investissement maternel différentiel directement lié à la qualité de la nourriture, soulignant le compromis mis en place par la femelle entre qualité et quantité d’œufs produits. Concernant les différentes nourritures testées pour évaluer la survie des larves, une nourriture à base de spiruline (*sera micron*) apparaît très adaptée aux premiers jours de croissance larvaire, avec une survie de prés de 70% au bout d’un mois. Cette nourriture semble pouvoir remplacer idéalement tout type de nourriture vivante dans des installations expérimentales.

The zebrafish, *Danio rerio* (Hamilton, 1822) has been widely used as a vertebrate model in scientific research and there are many advantages to its use in developmental and physiological studies, such as the transparency of the chorion, production of numerous eggs, a sequenced genome and no major rearing difficulties (Lawrence, 2007; Westerfield, 2000). Owing to these assets, the zebrafish has been recently proposed as a model for fish nutrition studies (Hardy, 2007). However, despite its popularity, there are relatively few studies on factors affecting the reproductive performance of the zebrafish, such as diet composition. Indeed, the diet delivered to the broodstock is likely to affect many aspects of fish physiology, making it difficult to compare studies. In addition, husbandry practices have been shown to alter reproduction in other fish species. For example, food delivered to parental *Cyprinus carpio* and *Oncorhynchus mykiss* had an important effect on the quality of the yolk and, consequently, egg viability (Watanabe, 1985). Particularly important is the dietary lipid content, which has major roles as an energy reserve and a membrane constituent (Meinelt *et al*., 1999; Tocher, 2003). Compared to the species described above, zebrafish are continuous spawners with a short vitellogenic period. Thus, the quantity and quality of food is likely to have an important role (Izquierdo *et al*., 2001), not only

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for female somatic growth, but also for providing sufficient energy stores for daily egg production.

In natural habitats, the diet of mature zebrafish comprises zooplankton (mainly crustaceans), insect larvae and algae (Lawrence, 2007; Spence *et al.,* 2007). Zooplankton and insect larvae are not easy to maintain in the laboratory and lose energy once dried. However, algal food has been proposed for some time to support the growth of omnivorous fish (Stanley and Jones, 1976). Among algae, the *Spirulina platensis,* which is a multicellular filamentous cyanobacterium from the family Phormidiaceae, appears to be a good candidate for feeding to adult fish (Güroy *et al*., 2012). This alga has been recommended for use in a food chain involving cichlids in a closed ecological recirculating aquaculture system (CERAS), to deliver food into outer space (Lu and Takeuchi, 2004). *S. platensis* is characterized by a high level of protein (> 60%), essential minerals, amino acids, lipids (6-11%) and vitamins (Oliveira *et al*., 1999; Babadzhavov *et al*., 2004). These properties make it a suitable food source for fish. Although the quantity of vitamins contained in *S. platensis* varies according to the season, it remains high throughout the year (Babadzhavov *et al*., 2004). These authors also showed that *S. platensis* contains many vitamins, including (mg/100 g unless otherwise stated): vitamin C (42.0-195.3), nicotinamide (0.6-5.3), B_1 (0.8-15.4), B2 (0.2-0.9), B_6 (0.3-4.0), B_c (0.2-0.6), B_{12} (0.3-0.8), β-carotene (64-140), vitamin E (10-19) and vitamin D (12 000 U). In addition, *S. platensis* has been found to be the best algal source of *n*-6 fatty acid (FA; 41.2% of total FA) (Algolina, Le Pouget, France) to date, especially of linoleic acid (LA) and γ-linolenic acid (GLA) (Qiang *et al*., 1997), which are not present in commercial flakes (Tocher *et al.,* 2001). Both are precursors of arachidonic acid (ARA), a major component in the formation of prostaglandin, which mediates steroidogenesis, oocyte maturation and ovulation (Pati and Habibi, 2002; Patiño and Sullivan, 2002). For example, surveys comparing the highly unsaturated FA (HUFA) composition of gonad tissues between spawning wild fish and captive broodstock often highlight the potential detrimental effect of insufficient dietary ARA on reproduction (Jaya-Ram *et al*., 2008).

The husbandry for developing zebrafish larvae is well established, and generally comprise the use of live food, such as paramecia, for the first few days of exogenous feeding, followed by the use of brine shrimp (*Artemia salina*) (Goolish *et al*., 1999; Lawrence, 2007). The main problem associated with such feeding is it maintenance alive in the laboratory. Some studies have examined larval survival as a function of diet. For example, ten artificial starter diets were tested, with survival rate varying between 10% and 84.3% at 21 days post fertilization (dpf; Goolish *et al*., 1999). The survival rate of zebrafish fry fed solely with dry food was approximately 12%, whereas a combined diet (live and dry food) resulted in a survival rate of 80% (Maley *et al*., 2007). Other authors reported a survival rate of 77% at 10 days (Simon *et al.,* 2011) and of 56% at 29 days using commercial food, and a survival rate of 86% of larvae fed on live food (Carvalho *et al*., 2006). However, in all three studies, none of the food contained algae.

Given the increasing number of studies involving *Spirulina* sp. as food for fishes, the impact of this alga on reproduction and larval survival needs to be evaluated. Therefore we designed a first experiment to document dietary effect of *S. platensis* on the reproduction performance rate (i.e. egg and larval size, mass, mortality and hatchability of fertilized egg, fecundity and number of spawn) of female adult zebrafish and a second experiment to document the effects of *S. platensis* on survival of larval fish.

MATERIALS AND METHODS

Zebrafish maintenance

Zebrafish were obtained from a commercial supplier (AquaSylva, Pertuis, France). Adult zebrafish were fed to satiation twice daily with commercial dry flakes (TetraMin®; comprising protein 47%, fat 10%, moisture 6%, fibre 3% and ash 11%) before the diet change and reared during an acclimatization period at 25 ± 1°C in aerated and dechlorinated tap water (pH 7.8). The photoperiodic regime was 12L:12D.

Experimental design

Sexual separation of adults was based on the anal fin covering the urogenital papillae in females and the yellow-gold coloration on the anal and caudal fins of males. After 10 days of sexual separation, two males and one female were put together to form a 'breeding group', and placed in a floating fish hatchery for 10 additional days. Two males were used to increase the probability of mating success. In addition, previous studies have shown that, in most cases, the ejaculate size of the males increases in the presence of rivals (Wedell *et al*., 2002). The experimental design comprised 20 plastic aquaria of 5 L each (ten aquaria per food treatment), inside of which placed a floating fish hatchery with one breeding group. Approximately 25% of the water volume was renewed daily.

Endpoints

Number, size of clutch and survival rate of eggs (%)

Throughout the 10 days of experimental period, the number of spawn was noted together with the number of eggs. To determine embryo development (live embryo/total number of eggs) x 100, eggs were collected 24 h after spawning to not jeopardize the sensitive stages that occur between cell cleavage and the completion of epiboly (Jones *et al*., 2009). Eggs were softly aspirated directly from the bottom of the tank using a tube, and transferred to a tank, and then into a Petri dish under a microscope for counting. All food wastes and feces were discarded and infertile oocytes and/or dead eggs were recorded and removed every day. These processes were carried out on a warm plate (26°C) to maintain the optimal temperature of egg medium.

Average egg mass and diameter

Ten eggs from each spawning event that had occurred during the first day of the experiment were placed in an aluminium pan and dried for two days at 55°C. The samples were weighed using an SE2 ultra-microbalance (precision of 0.1 μg, Sartorius, Gottingen, Germany). The diameter of 20 randomly selected eggs per dietary group was determined using a binocular microscope with camera (DS Camera Control Unit DS-U1, Nikon) and associated software (Visilog 6.6 viewer; Noesis).

Larval development

In total, 36 eggs per breeding group and per spawning event were kept in a triplicate of 20 ml glass beakers (three containers x 12 eggs) until all the eggs had hatched. Hatching rates were determined as the percentage of hatched eggs from live eggs at a given time. Three spawning events were followed: 1 day (d), 5 d and 10 d. The number of hatchings was counted three times a day for each 20 ml beaker for at least 6 dpf. As it was impossible to look after all the spawn from each breeding group, all eggs were considering fertilized at the point at which the light stimuli to begin reproduction was turned on.

The mean hatching time (HT_{50}), which represented the time necessary for 50% of the eggs to hatch, was calculated using a Log logistic model (see Statistical analysis for details) for each female. The time of hatching is not a good indicator of the embryo developmental stage (Kimmel *et al.,* 1995). However, this parameter was used, as it is relevant for detecting the effects of metal contaminants on the embryo (Fraysse *et al*., 2006; Bourrachot *et al*., 2008).

At 7 dpf, a sample of ten euthanised larvae from the spawn of the first day of each breeding group was dried (55°C) for 2 days and then weighed. In addition, two more larvae, collected in the same way, were measured (anterior-posterior length), resulting in 18 larvae measured for the flake group (FG; see below) and ten for the *S. platensis* group (SG; see below), because nine females from FG spawned the first day against fives females from SG. The 7-d survival rates of SG and FG larvae were also determined.

Experiment 1: effects of diet on the reproduction of adult fish

Diets

Based on the average weight of 40 males (306 ± 17 mg) and 20 females (450 ± 33 mg), fish were fed the equivalent of 5% of their body weight. Preliminary experiments showed that this amount is fully consumed by the fish and sustains regular reproductive events (Simon *et al*., 2011). Two types of food were used: pure *S. platensis* (*n*-6 FA, 41.2%; *n*-3 FA, 0.1%; MUFA, 13.5%; and saturated fatty acids, 44.9% of total fatty acids) from Algolina (Le Pouget, France), which formed SG (ten females; 20 males); and commercial flake food (TetraMin, *n*-3 FA, 11.3%; *n*-6 FA, 27.9%; MUFA, 30.6%; and saturated fatty acids, 30.3% of total fatty acids), which formed FG (ten females; 20 males). Food was delivered three times per day throughout the 20 days of the experiment.

Experiment 2: effects of diet on the survival rate of larval fish

For this experiment, 108 eggs coming from one spawn (breeding couple fed TetraMin Flakes) were collected at 24 h-post fertilization (hpf). Larval survival rate was studied as described above (three containers x 12 eggs). At 20 dpf, larvae were switched to a larger tube containing 100 ml tap water. Survival rates of the larvae were calculated every day from the 7th dpf based on three dietary conditions:

(i) Nano Reef CORAL FOOD given *ad libitum* (protein 52%, fat 14.5%, moisture 8%, fiber 1%, ash 12%, total HUFA ω-3: 30 mg g^{-1} dry weight and a DHA:EPA ratio higher than 2; particle size 30-150 μm);

(ii) pure *S. platensis ad libitum* (size 133 μm ± 89); or

(iii) Sera *Spirulina ad libitum*, where the major constituent is *Spirulina* sp. (Sera micron, protein 50.2%, fat 8.1%, moisture 5%, fiber 9.2%, ash 11.9%, particle size 62 ± 20 μm).

The food was distributed twice daily from 7 dpf to 14 dpf and, thereafter, three times per day.

Statistical analysis

Female weight and reproductive success were analysed using a Student's t-test since data distributions were normal. Egg viability and spawning success were analysed using a Chi-square test. Spawning frequency and spawning event as a function of the clutch size were analysed using the exact Fisher test (p values were adjusted using the Holm method). A generalized linear model [analysis of variance (ANOVA), Test 3 deviance analysis] was used to test the impact of both final wet weight and food quality on the reproductive success of female zebrafish. The HT_{50} was determined using a log-logistic model (Equation 1), which enabled us to consider all replicates within each group at each time. Data were compared using the exact Fisher test, and p values were adjusted using the Holm method.

Log logistic formula:

$y = 0 + [(1 - 0) / [1 + \exp(b(\log(x) - \log(e))]]$ [1],

where b is the slope of the curve around the point f inflexion (e); e is the time required to reduce hatching by 50% (HT_{50};

i.e. point of inflexion); *x* is the time and *y* is the response (hatching).

In all cases, the level of statistical significance was fixed at 5% ($p \leq 0.05$). All analyses were done using the statistical software 'R' (R 2.12.0, Development Core Team). Hatching time modelling was done using the add-on package 'drc' (Ritz and Streibig, 2005).

RESULTS

Experiment 1: effects of diet on the reproduction of adult fish

Broodstock and spawning information are detailed in table I. Only six of the ten SG females spawned, whereas all FG females did. In addition, total spawning events were more numerous for the FG females compared to SG females ($p = 0.013$, Chi-square test). Significantly fewer eggs were produced by the spawning SG females (81 ± 62, 178 ± 135; $p < 0.001$, Student's t-test). Moreover, after 20 days of food consumption, SG females were significantly lighter than FG females (Tab. I; $p = 0.016$, Student's t-test). Two of the four SG females that did not spawn died during the breeding period.

Both the final wet weight of females and the quality of the food significantly impacted the total number of eggs laid by each female (generalized linear model, final wet weight $p = 0.012$ and feeding treatment $p < 0.001$). Final wet weight explained 48% of the total deviance and 81% was explained when considering final wet weight together with diet quality.

During the first day of the breeding period, nine females spawned fed the FG diet and five fed the SG diet. However, considering the individual level within clutches, no differences occurred as a function of dietary treatment (Tab. II). Mean egg mass, egg diameter, and mass and length of larvae from the first spawn were similar, regardless of parental diet.

The distribution of clutch size (number of eggs and/or spawn) is represented in figure 1. A significant difference was observed between treatments ($p = 0.0003$, Fisher test); SG females spawned a significantly greater (33%) number of very small clutches (0-50 eggs) compared with the FG females (2%; $p = 0.007$, Fisher test). No significant difference for the other clutch sizes occurred between fish on the two kinds of diet.

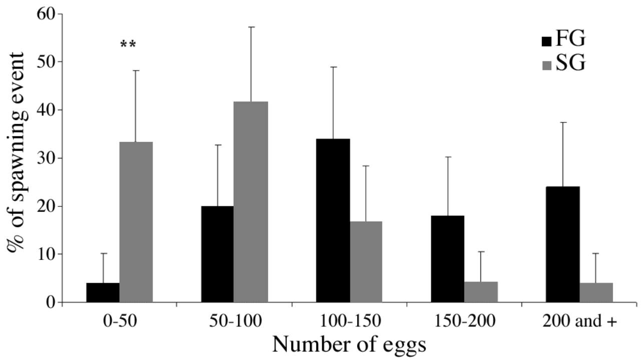

Figure 1. - Percentage of spawn according to the size of the clutch. Error bars represents standard error mean (SEM). **: p-value ≤ 0.01; FG: Flakes Group; SG: *Spirulina platensis* Group.

Following the first spawn, egg survival (determined at 24 h), was significantly higher from SG females (72%) compared with egg survival of FG females (38%) ($p < 0.001$, Chi-square test). When considering egg survival (determined at 24 h) during the whole experiment, the significant difference between groups was still observed with egg survival of 73% for the SG and 55% for the FG ($p < 0.001$, Chi-square test). However, there was no statistically significant difference in larval survival rate at 7 days between treatments.

HT_{50} was determined for eggs coming from spawn of day 1, day 5 and day 10 (Fig. 2). There were significant delays in the HT_{50} for SG eggs with FG eggs, with the former always hatching more readily than the latter. There was a delay of 3 h 20 min for eggs from spawn on day 1; a delay of 19 h 15 min for eggs from spawn on day 5; and a delay of 50 h 21 min for eggs from spawn of day 10 ($p = 0.001$, $p < 0.001$ and $p < 0.001$, respectively; F-Test). No difference in embryonic development stages between treatments was observed at the hatching period.

Experiment 2: effects of diet on the survival rate of larval fish

The survival kinetics of larvae fed with different diets is shown in figure 3. Food input started on the day 7 post-fertilization. Most larvae started to die at 13 dpf. At the end of

Table I. - Spawning performance of broodstock zebrafish maintained on *Spirulina platensis* or commercial flakes diets. *: p-value ≤ 0.05; ***: p-value ≤ 0.001.

	Spirulina platensis group SG	Flake group FG
Average number of spawn per female throughout 10 days	2.4 ± 0.8*	5 ± 0.63
Females that spawned, from a total possible of 10	6	10
Total number of eggs laid	1961	8899
Number of spawn	24	50
Fecundity	81 ± 62***	178 ± 135
Female weight (mg) after 20 days (n = 18)	396 ± 27*	482 ± 25

Table II. - Characteristics of eggs and larvae from the first spawn for each group of broodstock fed with different diets (FG: Flakes Group; SG: *Spirulina platensis* Group).

		Mean	SEM
Length (mm)	7 days larvae FG	3.69	0.05
	7 days larvae SG	3.68	0.04
Perimeter (mm)	Eggs FG	3.51	0.03
	Eggs SG	3.52	0.03
Weight (μg)	7 days larvae FG	85.5	2.20
	7 days larvae SG	86.2	3.30
	Eggs FG	107.0	4.00
	Eggs SG	111.0	6.00

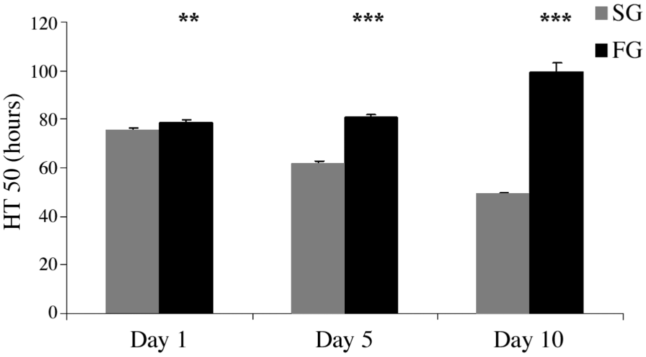

Figure 2. - HT50 represents the time required to observe 50% hatched embryos in the two diet groups spawned one (Day 1), five (Day 5) and ten (Day 10) days after diet change. **: p-value ≤ 0.01; ***: p-value ≤ 0.001. Standard errors are represented. FG: Flakes Group; SG: *Spirulina platensis* Group.

the experiment (24 dpf), larval survival using Sera *Spirulina* was 69%, 22% using Nano reef Coral food, and 11% using pure *S. platensis* ($p < 0.05$).

DISCUSSION

Effect of diet on broodstock reproduction

SG females lost weight (-13%) throughout the experiment (Tab. I), probably because of unbalanced nutrients and the low energy content of this algal food, which is usually used to complement other food sources (Belay *et al.*, 1996; Palmegiano *et al.*, 2005; Güroy *et al.*, 2012). Despite the lack of the added boost that stimulates food acceptance in FG, the form of *S. platensis* enabled the fish to consume the food efficiently. The small quantity of food supplied to the fish might have also contributed to the observed effects.

The results of the present study highlight the fact that both the quality and form of food can affect various reproductive endpoints. On the one hand, SG females produced significantly fewer spawn, delivering fewer eggs (x2.7) and smaller clutches than did FG females (Tab. I; Fig. 1). On the other hand, eggs laid by SG females displayed a higher viability rate (72% for the first spawn and 73% over the ten days) than eggs laid by FG females (38% for the first spawn and 55 % during the ten days). The difference in fecundity observed between the groups was partly explained (49%) by the final size of females (Tab. II). Several studies have shown that the number of egg laid is positively correlated with the size of female zebrafish (Spence and Smith, 2006; Uusi-Heikkilä *et al.*, 2010). Once the weight effect of the female was removed, a food effect was also noted, with SG females producing fewer eggs than expected, emphasizing a differential impact of food quality on the reproductive success of both groups. Life-history theory predicts that females in a poor environment should adjust their maternal investment by producing a few, large offspring. This pattern of investment has been described for female zebrafish (Uusi-Heikkilä *et al.*, 2010). However, the size of eggs differed according to the size of the female while the yolk size did not. In the present study, SG females produced only a few eggs, but the size of their first spawn did not differ from that of FG females (Tab. II). It has also been demonstrated that delivering different diets to zebrafish broodstock had no effect on the weight and diameter of the eggs produced (Markovich et *al.,* 2007). Interestingly, the present study revealed that survival rate was higher for eggs produced by SG females compared with eggs produced by FG females. This leads to examine two main hypotheses. In the first hypothesis, egg quality could be indicated by the nutrient components contained in the yolk, such as *n*-6 FA, rather than by egg size. FA egg composition was not measured here but an earlier survey indicated that the FA profile in the diet of broodstock and in the yolk were the same (Meinelt *et al.*, 1999). Nutrient components are a key factor affecting egg quality (Brooks *et al.*, 1997). Among them, GLA and ARA significantly improve egg quality (Bell and Sargent, 2003), larval survival rates and hatching rate of fish (Furuita *et al.*, 2003). The flakes used in the study did not contain as much *n*-6 FAs as *S. platensis*, providing a possible explanation for the discrepancy in egg survival results. The second hypothesis would involve sperm quality, which could also be influenced by dietary FA. Indeed, the quantity of *n*-6 FAs delivered to the broodstock has been shown to improve fertilization rate (Fernandez-Palacios *et al.*, 1997). In this study, it was observed for gilthead seabream *Sparus aurata* that the more ARA in the diet of the broodstock, the better the fertilization rate. This was possibly because of enhancement of sperm motility (Izquierdo *et al.*, 2001). Both hypotheses are not exclusive and in both cases, the preponderant role of the *n*-6 FAs is underlined.

The hatching time of eggs decreased with time (Fig. 2) for those laid by SG fish compared with FG fish. Concerning day 1, the significantly shorter hatching delay between treatments (3 h 20 min) could have been caused by a behavioral change at the start of reproduction, because SG females could have spawned earlier during the night than FG females.

However, when examining the hatching of eggs spawned on day 10, the significant difference between the HT_{50} of SG and of FG (50 h 21 m) was greater than 2 days. This large delay might have been because of an ontogenesis modification that accelerated the development process of the embryos and suggests that it was also the case for embryos from day 1 and day 5. The HT50 at day 1, day 5 and day 10 were 79 h, 81 h, 99 h and 75 h, 61 h, 49 h for respectively the FG and the SG. The HT50 of reference is 72 h (Kimmel *et al.,* 1995), which strengthens the hypothesis of an acceleration of the development for embryos of the SG group. In addition, images taken over 24 h showed that most eggs produced by SG females were at a more advanced stage of development than those from FG females. The high protein level contained in the food of the broodstock is known to impact the ontogeny of the larval fish by accelerating the development process (El-Sayed *et al.*, 2003). Thus, the large amount of protein contained in *S. platensis* might have impacted the ontogeny of zebrafish embryos. Moreover, vitamins and carotenoids might also have a major role in larval development, because they can enhance protein stability (Izquierdo *et al.*, 2001; Regunathan and Wesley, 2006). In addition, among carotenoids, the β-carotene (provitamin A) was shown to be essential for zebrafish embryogenesis (Lampert *et al.*, 2003). Overall, the present results suggest that FG females, which loosed weight during this experiment, adjusted their reproductive investment possibly by providing a part of their own metabolic stores. This would have led to an increase in egg quality (i.e. high survival) compared with FG females. Future experiments should focus on the amount of *S. platensis* delivered solely or as a supplement to promote growth rate.

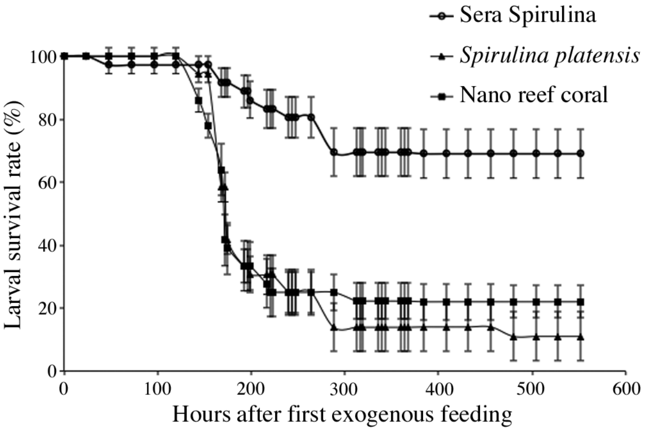

Figure 3. - Larvae survival rate (mean +/- SEM) when larvae are fed with 3 different diets, starting from 7 days post fertilization (0 hour).

Effect of food on larval survival rate

It is well established that the larval stage is one of the most sensitive periods for both marine and freshwater fishes, mainly because fishes of both environments have a deficient immune system during the first few weeks of life (Belay *et al.*, 1996). *S. platensis* has been described as an excellent candidate for accelerating the development of the immune system (Babadzhavov *et al.*, 2004) and for providing resistance against bacterial infection in Masou salmon (*Onchorynchus masou*) and ayu (*Plecoglossus altivelis*) (Kato and Miyakawa, 1992). Except for larvae fed with the Sera *Spirulina* food, all larvae started to die at approximately 13 dpf (Fig. 3). This suggests that this is a crucial time in larval development, at which larvae that died were not able to capture the delivered food. Although Nano Reef Coral food appeared to provide sufficient energy for some larvae, the survival rate (approximately 20%) was low. Given that the size of these particles was adapted to the mouths of young larvae (150-250 μm) and the colour (orange) was attractive, the quality of the diet, which contained a high level of HUFA adapted for marine fishes, did not meet zebrafish dietary requirements. In cichlids, high dietary HUFA levels have also been reported to cause lipid peroxidation and reduce growth rate (Huang *et al.*, 1998; Ling *et al.*, 2006). Larvae fed solely on *S. platensis* displayed a low survival rate (approximately 15%), which might have been the result of both the low attractiveness and the low availability of this crushed food, which appeared to dissolve quickly. Thus, it is probable that most of the larvae were not able to catch it readily. Conversely, individuals fed the Sera *Spirulina* diet displayed a higher survival rate (69%), which might have been because the *Spirulina* sp. in this case was encapsulated and associated with other food compounds. Thus, this diet had all the advantages (colour, availability, size and quality) to support efficient larval growth. *Spirulina* inclusion in food delivered to juvenile sturgeon (*Acipenser baeri*) has also been shown to improve growth rate, feed conversion rate and protein efficiency compared with control fish (Palmegiano *et al.*, 2005).

Diets in aquaculture are usually based on conventional feedstuffs, such as fish oil and fish meal, that are expensive. The future development of aquaculture is likely to be constrained by the availability of an alternative source of ingredients (Palmegiano *et al.*, 2005) with a vegetable origin, such as algae. Here, we have provided an overview of the positive and the negative endpoints of using *S. platensis* as food for female adult and larval zebrafish compared with other conventional foods. Although it might be feasible to rear zebrafish without using live food and using Sera *Spirulina* food instead, our findings show that such a diet will only be appropriate for the crucial first 20 days of exogenously feeding larvae and a switch to a more commonly commercial diet is recommended afterwards. Our experiments originally aimed to evaluate the husbandry of zebrafish larvae in laboratory studies (e.g. toxicology) and were therefore carried out only in small containers. Hence, even they bring new interesting data on dietary effect on zebrafish reproduction in small containers, their application to large commercial scale aquaculture are still to be developed.

Acknowledgments. – This work was supported by the Institute for Radioprotection and Nuclear Safety in France. We especially thank Iban Seiliez, Stéphane Panserat, Elise Dufour, Claire Della Vedova, Sergio Polakof, Rodolphe Gilbin and Martin Walton for improvements to the manuscript and for English language corrections. We also would like to acknowledge two anonymous reviewers who greatly contributed to the improvement of this manuscript.

Disclosure statement. – No competing financial interest exists.

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